

Claims

1. Method for hybridisation of probes onto immobilized genomic DNA comprising the steps of:
 - (a) providing intact genomic DNA and denaturing said intact genomic DNA;
 - (b) immobilizing said denatured intact genomic DNA onto a matrix; said matrix comprising pore sizes within a range of 0.6 μm to 2 μm including the outer limits;
 - (c) providing a set of probes and passing said probes through said matrix under conditions favouring hybridisation of the probes to its complementary sequence in said intact genomic DNA; and
 - (d) washing off non-hybridised probes through said matrix, leaving formed hybridised intact genomic DNA/probe complexes for further analysis.
2. Method according to claim 1, wherein said denatured intact genomic DNA is permeated within said matrix.
3. Method according to any of claims 1 or 2, wherein said probes are passed through said matrix by at least one cycle of alternating downwards and upwards flow.
4. Method according to any of claims 1 to 3, wherein said washing step is carried out by passing through said matrix a wash fluid by at least one cycle of downwards flow.
5. Method according to any of claims 1 to 4, wherein said matrix is a membrane.
6. Method according to claim 5, wherein said membrane comprises a 3D network structure.
7. Method according to claim 6, wherein said network structure is a flow-through structure.
8. Method according to claim 6 or 7, wherein said network structure is a fibre network structure.
9. Method according to claim 8, wherein said fibre is of vegetable origin.

10. Method according to claim 9, wherein said fibre is cellulose.
11. Method according to any of claims 1 to 10, wherein the matrix allows for a flow rate comprised between 50mm/30min and 250mm/30min including the outer limits.
12. Method according to any of claims 1 to 11, wherein said matrix is activated with an affinity conjugate.
13. Method according to claim 12, wherein said affinity conjugate is chosen from the group comprising poly-L-lysine, poly-D-lysine, 3-aminopropyl-triethoxysilane, poly-arginine, polyethyleneimine, polyvinylamine, polyallylamine, tetraethylenepentamine, ethylenediamine, diethylenetriamine, triethylenetetramine, pentaethylenhexamine and hexamethylenediamine.
14. Method according to claim 13, wherein said affinity conjugate is poly-L-lysine.
15. Method according to any of claims 1 to 14, wherein said probes are flanked by primer binding sequences.
16. Use of a method according to any of claims 1 to 15 for intact genomic DNA hybridisation.
17. Use of a method according to any of claims 1 to 15 for detection and quantification of target nucleic acids in an intact genomic DNA sample.
18. Method for target nucleic acid detection and quantification in an intact genomic DNA sample comprising the steps of:
 - (a) providing intact genomic DNA and denaturing said intact genomic DNA;
 - (b) performing a hybridisation according to a method as described in any of claims 1 to 15;
 - (c) recovering hybridised probes; and essentially simultaneously amplifying any recovered probe using a single primer pair, each member of said primer pair binding to each recovered probe onto the respective flanking primer binding sequences of said probe; and,

(d) qualitatively and quantitatively analysing the recovered amplified probes of step (c).

19. Method according to claim 18, wherein the analysis of step (d) is by microarray analysis.
20. Method according to claim 18 or 19, wherein each probe is flanked 5' and 3' by primer binding regions with said 5' and 3' flanking primer binding sequences being the same or substantially the same for each probe.
21. Method according to any of claims 18 to 20, wherein said amplification of step (c) is a quantitative amplification.
22. Method according to claim 21, wherein said amplification is by means of polymerase chain reaction.
23. Method according to any of claims 18 to 22, wherein the amplified probes are provided with a label.
24. Method according to claim 23, wherein said label is a fluorescent label.
25. Use of a method according to any of claims 18 to 24 for genomic screening.
26. Use of a method according to any of claims 18 to 24 for detecting deletions or duplications in genomic DNA.
27. Use of a method according to any of claims 18 to 24 for genome profiling.
28. Use of a method according to any of claims 18 to 24 for identifying and quantitatively detecting the degree of pathogenesis, disease or contamination in a sample.
29. Use of a method according to any of claims 18 to 24, for identifying and detecting the presence of infectious agents in a sample.
30. Use of a method according to any of claims 18 to 24, for genotyping pathogens present in a sample.

31. Device for flow-through hybridisation of probes onto immobilized intact genomic DNA comprising a well holder, said well holder comprising one or more round wells with a fixed diameter, said wells exposing a fibre network matrix, said matrix comprising pore sizes within a range of 0.6 μm to 2 μm including the outer limits; wherein said matrix permits immobilization of intact genomic DNA and which allows hybridisation of said immobilized intact genomic material with probes by flow-through hybridisation.
32. Device according to claim 31, wherein said matrix permits permeation of intact genomic DNA.
33. Apparatus for flow-through hybridisation of probes onto immobilized genomic DNA comprising:
- (a) a device according to claim 31 or 32;
 - (b) means for addition of a controlled amount of fluid to at least one of the wells of the device as described in (a);
 - (c) means for applying and/or maintaining a controlled pressure difference over the matrix in each of the wells.
34. Kit for flow-through hybridisation of probes onto immobilized intact genomic DNA comprising :
- (a) a device according to claim 31 or 32; and
 - (b) instructions to carry out a method according to any of claims 1 to 15 or 18 to 24.
35. Kit according to claim 34, additionally comprising:
- (a) a set of probes, wherein each probe is flanked 5' and 3' by primer binding regions with said 5' and 3' flanking primer binding sequences being the same or substantially the same for each probe;
 - (b) a single primer pair, each member of said pair being complementary to a primer binding region;
 - (c) optionally amplification components allowing the amplification of any recovered hybridised probe; and
 - (d) optionally a microarray, said microarray allowing analysis of the hybridisation results obtained by a method according to any of claims 1 to 15 and 18 to 24.